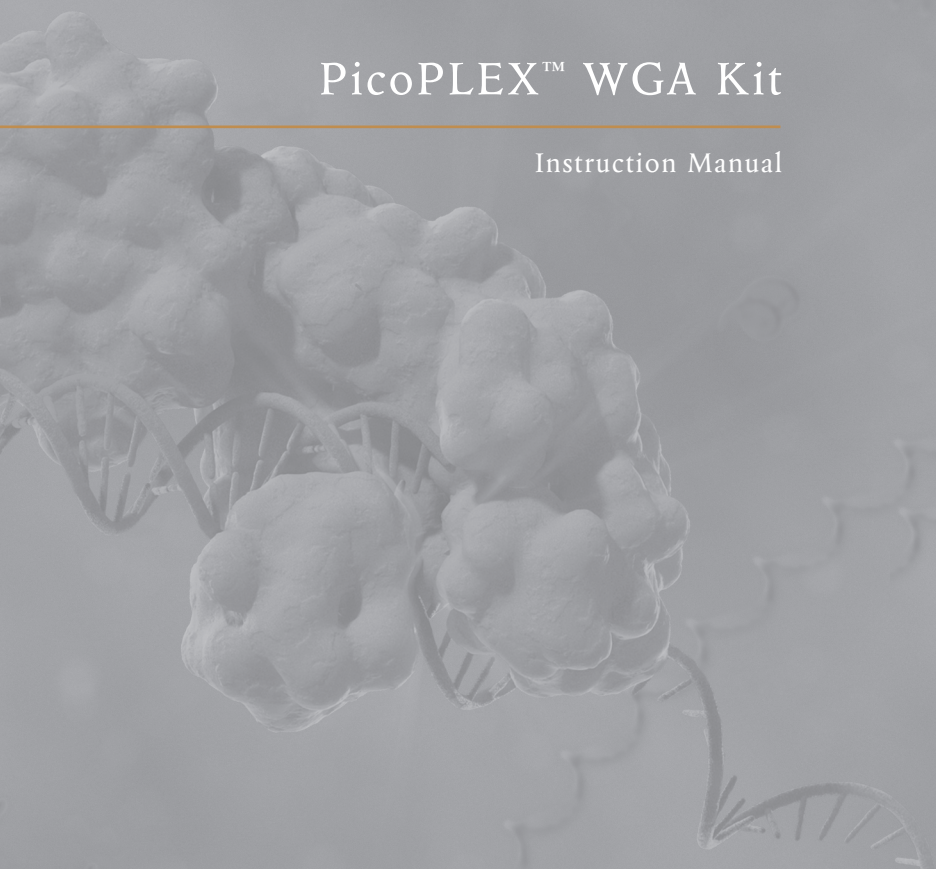
POLYMERASES & AMPLIFICATION

PicoPLEX™ WGA Kit

Instruction Manual



NEB #E2620S/L
12/50 reactions

Developed and manufactured by Rubicon Genomics, Inc.
Distributed by New England Biolabs, Inc.

RUBICON GENOMICS

NEW ENGLAND BioLabs® Inc.

Table of Contents:

Kit Components:

The PicoPLEX WGA Kit contains a sufficient supply of cell extraction components and amplification reagents to perform 12 whole genome amplification reactions (small) or 50 whole genome amplification reactions (large).

	#E2620S	#E2620L
Cell Extraction Buffer	0.1 ml	0.3 ml
Extraction Enzyme Dilution Buffer	0.1 ml	0.3 ml
Cell Extraction Enzyme	0.005 ml	0.015 ml
Pre-Amp Reaction Mix	0.1 ml	0.275 ml
Pre-Amp Enzyme	0.005 ml	0.015 ml
Amplification Reaction Mix	0.35 ml	1.4 ml
Amplification Enzyme	0.012 ml	0.05 ml
Nuclease-Free Water	1.8 ml	1.8 ml

Note: Pre-Amp Reaction Mix and Amplification Reaction Mix contain all components necessary for the reactions except enzyme. Add enzyme to these mixes at the appropriate time as described in the protocols.

Storage Information:

Store the PicoPLEX WGA Kit at –20°C for up to 12 months from the assay date. The expiration date can be found on the box label.

Transfer the Cell Extraction Enzyme, Pre-Amp Enzyme and Amplification Enzyme tubes to ice just before use. All other components can be thawed on ice and briefly vortexed prior to use.

This kit is designed to amplify picogram quantities of DNA, therefore extreme caution must be exercised to prevent introduction of exogenous DNA. Good laboratory practices for PCR should be followed when storing and handling reagents as well as when setting up reactions.

Required Materials Not Included

PCR thermal cycler (Recommended: Real-time qPCR instrument)

PCR tubes or 96-well plates for cell extraction

PCR plate seals for pre-amp steps (Recommended: Microseal® 'F' Foil film, Bio-Rad Cat. #MSF-1001 or Axymat silicone sealing mats, Axygen Cat. #AM-96-PCR-RD)

PCR optically clear plate sealing film for amplification steps (Recommended: Microseal "B" film, Bio-Rad Cat. #MSB-1001)

Low Binding Barrier Filter pipette tips

Note: Considerable (> 5 µl) evaporation may occur during step 6 of the Pre-Amplification Protocol if the incubation is being performed in a PCR tube or plate without a tight seal. Evaporation may reduce the robustness and reproducibility of the PicoPLEX WGA Kit. A mock step 6 incubation from the Pre-Amplification Protocol using 15 µl of water is recommended to confirm the suitability of the selected tube or plate/seal combination.

PBS buffer for cell washes (Recommended: 20X PBS, Cell Signaling Technology Inc. Cat. #9808 or 10X PBS, USB Corporation Cat. #75889)

Additional Optional Materials Not Included

DNA purification system (Recommended: DNA Clean & Concentrator™-5 Kit, Zymo Research Cat. #D4014 or MultiScreen PCR_{96} Plate, Millipore Cat. #MSNU03050 or QIAquick PCR Purification Kit, Qiagen #28104)

SYBR Green I dye (Recommended: Invitrogen Cat. #S7563)

Note: Dye can be diluted to an intermediate concentration in DMSO for storage at –20°C. For the final working stock, this intermediate stock can be further diluted in 1X TE (pH 8.0) for storage at 4°C for up to 1 month.

Introduction:

The PicoPLEX WGA Kit, developed and manufactured by Rubicon Genomics, Inc., efficiently amplifies total DNA from single cells or their DNA equivalent about 1 million-fold to produce 2–5 micrograms of amplified DNA in under 3 hours. The kit can also be used successfully in situations where the low input amount is uncertain as it works well over a range of inputs up to thousands of cells or several ng DNA to achieve this final yield of amplified DNA. Both AT- and GC-rich regions are represented reproducibly using this kit. With single blastomere and polar bodies, the kit is able to achieve 95% amplification success rate. The kit performs reproducibly and generates greater than 90% correlation coefficients for qPCR Ct data from replicate single-cell reactions. This method features single-copy sensitivity as well as high specificity with an expected 5 PCR cycle delay between experimental samples and non-template negative controls. The PicoPLEX WGA Kit produces amplified DNA fragments suitable for Copy Number Variation (CNV) analysis using oligonucleotide aCGH or qPCR; SNP genotyping, mutation detection and sequencing.

Cell Specifications:

Cell Types

Single blastomeres, polar bodies, trophoblastic cells, amniocytes and cultured cells have been amplified by the PicoPLEX WGA Kit. For the most reproducible results with cultured cells, homogeneous cell lines with stable karyotypes are recommended.

Number of Cells

The PicoPLEX WGA Kit produces the same robust, reproducible amplification results from a single cell or its DNA equivalent as from large numbers of cells (e.g., 1,000 cells).

Note: To avoid stochastic variation that can occur when performing large dilutions of DNA it is recommended that controls approximating single cells contain 15 pg DNA, an amount equivalent to two diploid cells. Although the PicoPLEX WGA Kit can be used to amplify multiple cells, this kit provides unique performance advantages with single cell samples.

Cell Collection Methods

Flow sorting, dilution, and micromanipulation are collection methods that are compatible with the PicoPLEX WGA Kit. Cell staining may negatively affect kit performance. Formalin fixation must be avoided if optimal results are to be achieved.

Washing Cells

Cell washing is strongly recommended to minimize non-cellular DNA contamination of the cell preparation. Mg^{2+}-free, Ca^{2+}-free, BSA-free PBS may be used for washing at a 1X concentration.

Wash buffers containing Mg^{2+}, Ca^{2+} or BSA must be avoided.

The PBS volume carried over with the cell sample into the Amplification Protocol **cannot exceed 2.5 µl**.

Amplification of Control DNA:

Control DNA samples are useful references for some analytical platforms such as microarrays and qPCR. WGA-amplified samples should be compared to WGA-amplified control DNA rather than to un-amplified control DNA for the most accurate results.

Control DNA samples must be prepared according to the Sample Preparation Methods and amplified using the procedure specified in this protocol (see page 6).

Analyzing Amplification Efficiency:

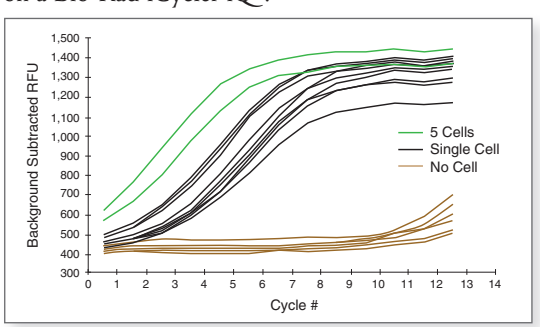
Sample amplification efficiencies can be analyzed by performing the amplification reactions with SYBR® Green I in a real-time qPCR instrument. During the amplification reaction, double-stranded amplified molecules are bound by the non-sequence-dependent SYBR Green I dye and the accumulation of amplified product is detected as an increase in fluorescence by the real-time instrument.

Data analysis should be performed on raw background-subtracted (not baseline cycle normalized) fluorescence and the instrument/software should be set to the appropriate mode.

Amplification curves will have a similar appearance for all single-cell WGA reactions with an immediate 8–9 cycle upward sloping phase, followed by a relatively flat "plateau" phase (Figure 1).

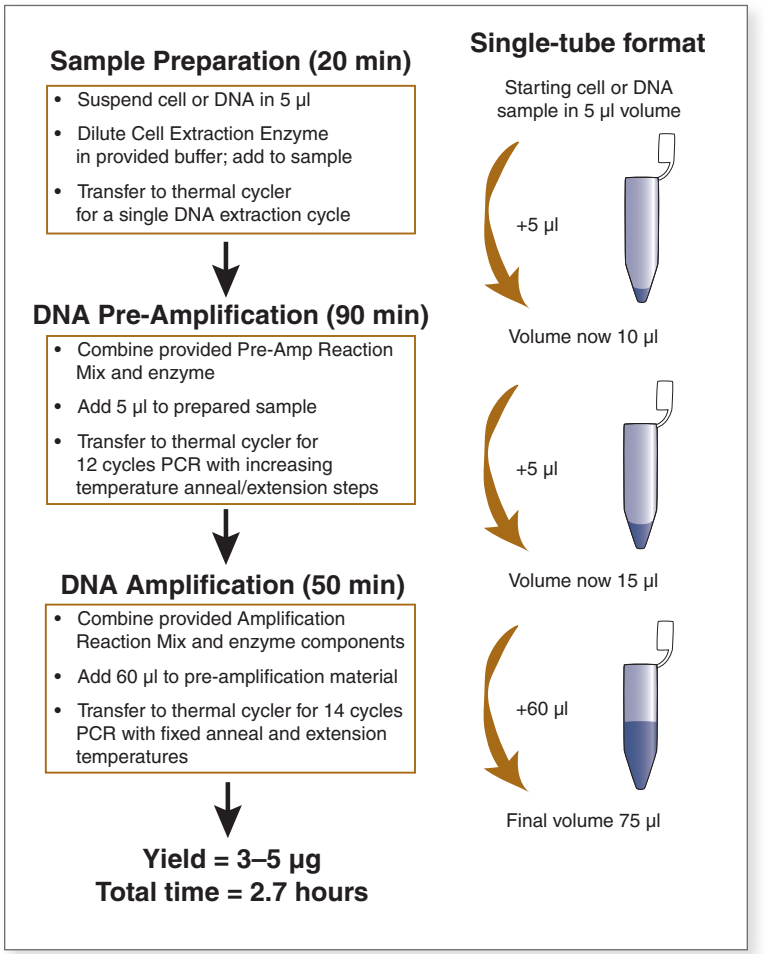
Non-template control amplification curves are delayed (right-shift) by 5–6 PCR cycles compared to single-cell amplification curves. A smaller delay of control curves may indicate DNA contamination introduced with the sample or during the WGA process.

Figure 1: Example of Background Subtracted RFU amplification curves for replicate single-cell and control no cell WGA reactions that were monitored on a Bio-Rad iCycler iQ®.



Data provided by Rubicon Genomics, Inc.

Figure 2: Workflow overview for a single cell or 15 pg-15 ng DNA



(for detailed instructions please refer to pages 6–8)

Sample Preparation Methods:

Cell Sample (5 µl)

1. Wash or dilute cells with 1X PBS buffer, according to the recommendations in the Cell Specifications section of this manual (see page 3).
2. If collecting cells by flow sorting: Collect a single cell into 5 µl of Cell Extraction Buffer in a PCR tube or well.

   If collecting cells by micromanipulation or dilution: Transfer a single cell in minimal 1X PBS volume (< 2.5 µl) to a PCR tube or well containing an appropriate volume of Cell Extraction Buffer to achieve a total cell sample volume of 5 µl.
3. Immediately freeze and store cells at –80°C or proceed directly to the Pre-Amplification Protocol (see below).

Control DNA Sample (5 µl)

1. Prepare a 1 ng/µl purified DNA solution in a PCR tube or well by diluting a control DNA stock with 5 mM Tris-HCl (pH 8.0).
2. Vortex the 1 ng/µl DNA solution for 30 seconds.
3. Add 3 µl of the 1 ng/µl DNA solution to 197 µl of 5 mM Tris-HCl (pH 8.0) to prepare a 15 pg/µl DNA sample.
4. Vortex the 15 pg/µl DNA solution for 30 seconds.
5. Add 1 µl of the 15 pg/µl DNA solution to 4 µl of Cell Extraction Buffer in a PCR tube or well.

Pre-Amplification Protocol:

1. Combine Extraction Cocktail components and mix well:

EXTRACTION COCKTAIL	VOLUME PER 5 SAMPLES
Extraction Enzyme Dilution Buffer	24 µl
Cell Extraction Enzyme	1 µl
Total Volume	25 µl

2. Add 5 µl of freshly-prepared Extraction Cocktail to each 5 µl cell sample or control DNA sample prepared by the sample preparation methods.
3. Incubate sample in a thermal cycler as follows:

CYCLES	TEMP	TIME
1	75°C 95°C 12°C	10 minutes 4 minutes Hold

4. Combine Pre-Amp Cocktail components and mix well:

PRE-AMP COCKTAIL	VOLUME PER 5 SAMPLES
Pre-Amp Reaction Mix	24 µl
Pre-Amp Enzyme	1 µl
Total Volume	25 µl

5. Add 5 µl of Pre-Amp Cocktail to each cell or control sample.
6. Incubate sample according to the thermal cycler program below:

CYCLES	TEMP	TIME
1	95°C	2 minutes
12	95°C 15°C 25°C 35°C 65°C 75°C	15 seconds 50 seconds 40 seconds 30 seconds 40 seconds 40 seconds
1	4°C	Hold

7. Briefly centrifuge sample and place Pre-Amp incubation product on ice.

Amplification Protocol:

1. Combine the following Amplification Cocktail components and mix well:

AMPLIFICATION COCKTAIL	VOLUME PER 5 SAMPLES
Amplification Reaction Mix	125 µl
Amplification Enzyme	4 µl
Nuclease-Free Water	171 µl
Total Volume	300 µl

Note: Sample amplification efficiency may be analyzed using a real-time qPCR instrument by adding SYBR Green I Dye at 0.125X final concentration in the Amplification Cocktail (see page 2). Some instruments require additional dyes for signal normalization.

2. Mix 60 µl of the freshly prepared Amplification Cocktail with the 15 µl pre-amp incubation product and mix gently by pipet.
3. Amplify sample according to the thermal cycler program below:

CYCLES	TEMP	TIME
1	95°C	2 minutes
14	95°C 65°C 75°C	15 seconds 1 minute 1 minute

Note: 14 cycles are recommended based on testing performed with flow-sorted cultured cells. Some cell types may require up to 16 cycles to obtain maximal yields.

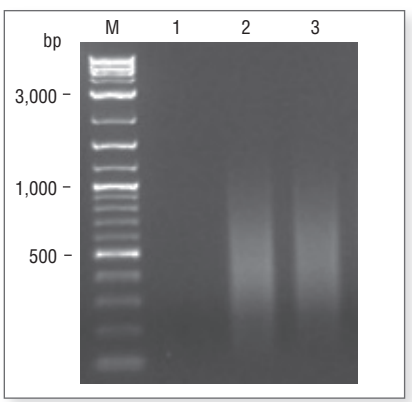
4. Immediately store the amplified product at –20°C or purify.

   Note: Many applications require purifying and quantifying WGA products before use. Products amplified using this kit can be purified with spin columns or filter plates. See page 2 for recommended purification systems.

5. Quantitate purified amplification products by UV absorbance (1 OD_{260} = 50 µg/ml). Store the purified amplification product at –20°C.

   Note: PicoGreen™ or other double-stranded specific methods for DNA measurement will not give reliable product concentrations. The amplified material will be a mixture of predominantly double-stranded material with some single-stranded material present.

Figure 3: Gel image of reaction products.



The size range of amplified product from a sorted single cell or 15 pg DNA (diluted human genomic DNA equivalent to two diploid human cells; see note on page 3). 10 µl of 75 µl reactions were analyzed by 1.4% agarose gel electrophoresis: Lane 1: no cell/DNA negative control, Lanes 2 and 3: independent reactions using 15 pg human genomic DNA. Note: The appearance of amplified product will be identical whether the starting material is a single cell or this amount of DNA. Marker M is 1 µg 2-Log DNA Ladder (NEB #N3200).

Troubleshooting Guide:

Observation	Probable Cause(s)	Solution(s)
No amplified product.	Sample tube or well did not contain a cell.	Confirm that cell collection method reproducibly results in single cell per tube or well.
	Improper sample preparation.	Follow instructions in Cell Specifications (page 3).
	Improper purification or quantification.	Follow instructions for purifying and quantifying amplification products (page 6).
Single cell amplification curve reaches "plateau" phase earlier than the 15 pg control DNA reaction.	Greater than one cell per sample.	Confirm that cell collection method reproducibly results in single cell per tube or well.
	Single cell sample is contaminated with exogenous DNA.	Use fresh, BSA-free PBS.
Non-template control amplification curve appears early or produces yield similar to amplification curve of the experimental sample.	Control solution is contaminated with DNA.	Use fresh control solution.
	Work area is contaminated with DNA.	Clean area thoroughly and use PCR-dedicated plastics and pipettes.
	Kit has become contaminated with DNA.	Use fresh kit.

For more information contact info@neb.com or visit www.neb.com

Frequently Asked Questions (FAQs):

How many cells can be amplified by the PicoPLEX WGA Kit?

The same robust, reproducible amplification is obtained from single cells as from 1,000 cells, but the greatest advantage over other WGA methods are obtained with single cells.

Does the PicoPLEX WGA Kit amplify genomic loci?

Yes, with a ~90% correlation coefficient for qPCR C_t data from replicate single-cell reactions.

Are all genomic loci equally represented in products amplified from the PicoPLEX WGA Kit?

No, WGA products have reproducibly high representation for both alleles of 70–90% of genomic loci and reproducibly low representation for the remainder of loci.

Does the PicoPLEX WGA Kit amplify GC-rich regions?

Yes, the kit has been optimized to amplify GC-regions with excellent representation but also works well with AT-rich regions.

Can negative control reactions not containing a cell or DNA be distinguished from single cell reactions?

Yes, while the PicoPLEX WGA Kit amplifies with single-copy sensitivity and high specificity, there will be at least a 5 PCR cycle delay between WGA of single cells and WGA of negative control buffer reactions.

How robust is the WGA process?

The PicoPLEX WGA Kit has a ~95% amplification success rate with flow-sorted tissue culture cells, limited by the uncertainties of sorting rather than amplification. Single cells always give a high, reproducible yield of amplified genomic DNA. Negative controls should always give virtually no background.

How rapid is the Single Cell WGA process?

The entire process, from cell lysis to DNA amplification yields of 2–5 µg, is < 3 hours.

How long are the expected amplicons generated with the PicoPLEX WGA Kit?

The WGA kit generates a pool of amplicons, with the majority of the amplified DNA ranging from 200–1000 bp in length with an average length of 500 bp.

Should cells be washed before collections?

Yes, cells should be washed to minimize extraneous DNA or growth media contaminants. We recommend washing in PBS (Ca^{2+}-Free, Mg^{2+}-Free, and BSA-Free) and limiting carry-over of wash buffer with each cell to less than 2.5 µl.

Which cell collection methods are compatible with the PicoPLEX WGA Kit?

Flow sorting, dilution and micromanipulation.

Are there special requirements for flow sorting?

Yes, we strongly recommend not fixing or staining the cells and light scattering or phase contrast should be used to sort or collect.

What cells types have been successfully amplified by the PicoPLEX WGA Kit?

Single blastomeres, polar bodies, trophoblasts, amniocytes, and cultured cells.

Can the PicoPLEX WGA Kit be used with subcellular materials?

Yes; the kit works well with sub-cellular (chromosomal) genomic preparations and with low level DNA preparations.

Can the PicoPLEX WGA Kit be used with bacterial cells?

Limited testing has successfully been done with gram negative bacteria, but is less well characterized.

For more information contact info@neb.com or visit www.neb.com

Ordering Information

PRODUCT	NEB #	SIZE
PicoPLEX WGA Kit	E2620S/L	12/50 rxns
COMPANION PRODUCTS		
Gel Loading Dye, Blue (6X)	B7021S	6.0 ml
Gel Loading Dye, Orange (6X)	B7022S	6.0 ml
Quick-Load® 100 bp DNA Ladder	N0467S/L	125/375 lanes

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